Synthesis of 4-alkyl-, 4-aryl- and 4-arylamino-5-aminoisoquinolin-1-ones and identification of a new PARP-2 selective inhibitor

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The considerable interest in substituted isoquinolin-1-ones related to 5-aminoisoquinolin-1-one (5-AIQ) as drugs points to a need for an efficient and straightforward synthesis of the 4,5-disubstituted bicycles. Bromination of 5-nitroisoquinolin-1-one gave 4-bromo-5-nitroisoquinolin-1-one but neither this nor 5-amino-4-bromoisoquinolin-1-one would participate in Pd-catalysed couplings. Protection of the lactam as 1-methoxy- and 1-benzyloxy-4-bromo-5-nitroisoquinolines, however, permitted Stille, Suzuki and Buchwald-Hartwig couplings to take place in high yields, insensitive to electronic demands and severe steric bulk in the arylboronic acids. Lithiation of 4-bromo-1-methoxy-5-nitroisoquinoline and quench with iodomethane gave 1-methoxy-4-methyl-5-nitroisoquinoline in low yield. Demethylation of the 1-methoxy-4-substituted-5-nitroisoquinolines with hydrogen bromide gave 4-substituted-5-nitroisoquinolin-1-ones, whereas hydrogenolytic debenzylation was achieved with simultaneous reduction of the 5-nitro group. 5-Amino-4-(4-trifluoromethylphenyl)isoquinolin-1-one was identified as a new potent and selective inhibitor of poly(ADP-ribose)polymerase-2 (PARP-2).

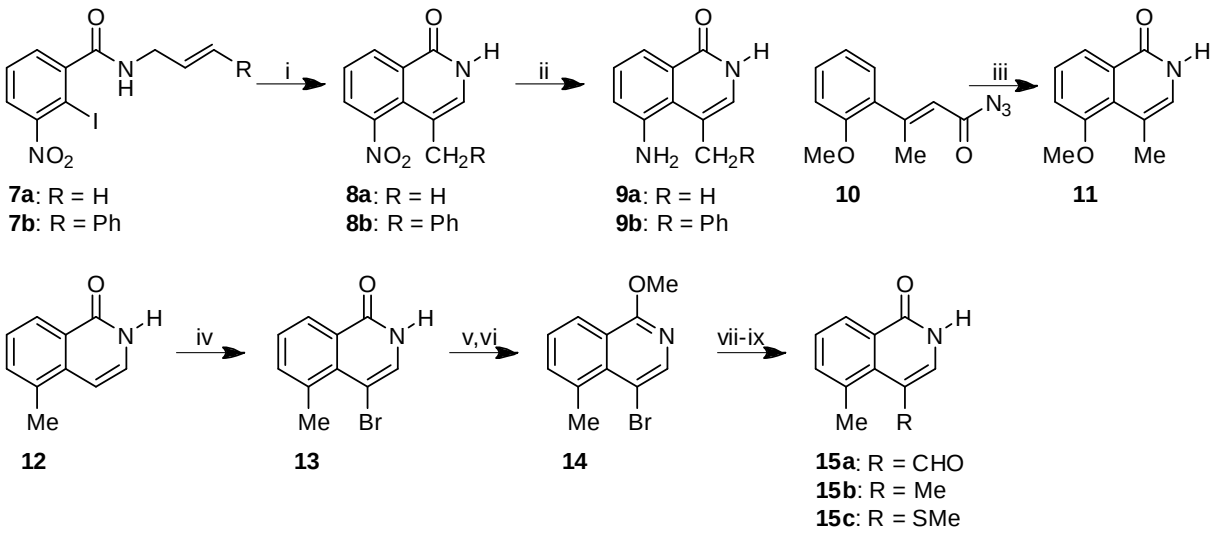
Introduction

Isoquinolin-1-ones are of considerable interest as potential drugs, particularly the 5-substituted analogues, which are potent inhibitors of poly(ADP-ribose)polymerases (PARPs). Simple 5-substituted isoquinolin-1-ones and 5-substituted 3,4-dihydroisoquinolin-1-ones were reported as long ago as 1991 to be potent inhibitors of PARPs *in vitro* and in cells[1,2] and 5-hydroxyisoquinolin-1-one **1** (often mis-named as the tautomer 1,5-dihydroxyisoquinoline **2**, Figure 1) has shown good inhibition of the enzyme *in vitro* and in models of inflammation and other PARP-mediated diseases *in vivo*.[3] However, of this series, it is the 5-amino analogue 5-AIQ **3** which has shown most promise as an inhibitor of PARPs, partly owing to the exceptional solubility in water of its hydrochloride salt. 5-AIQ **3** is active in models *in vivo* and *in vitro* of a wide range of disease states, including colitis,[4] ischaemic heart disease,[5] haemorrhagic shock[6] and spinal cord trauma,[7] and has recently been shown to have strong antimetastatic effects in a murine model of cancer.[8] We have recently reported[9] that 5-benzamidoisoquinolin-1-ones **4** and one 3-substituted 5-benzamidoisoquinolin-1-one **5** are selective inhibitors of the PARP-2 isoform; 5-benzoyloxyisoquinolin-1-one **6** also selectively inhibits PARP-2.[10]

In the light of these biological activities, we wished to explore 4,5-disubstituted isoquinolin-1-ones. There is a marked paucity of reports of preparation of such compounds in the chemical journal literature, shown in Scheme 1. We have disclosed recently that palladium-catalysed cyclisation of N-allyl-2-iodo-3-nitrobenzamide **7a** at high-temperature gives 4-methyl-5-nitroisoquinolin-1-one **8a** in low yield; similar reaction of N-cinnamyl-2-iodo-3-nitrobenzamide **7b** leads inefficiently to 4-benzyl-5-nitroisoquinolin-1-one **8b**.[11] These products can be then reduced readily to their 5-amino analogues **9a,b**. Croisy-Delcey *et al*. achieved the synthesis of 5-methoxy-4-methylisoquinolin-1-one **11** in moderate yield by Curtius rearrangement / thermal cyclisation of the acyl azide **10**.[12] Sercel *et al*. prepared 4-bromo-5-methylisoquinolin-1-one **13** by bromination of 5-bromoisoquinolin-1-one **12** with pyridinium perbromide; this was converted to the lactim **14** and from this they were then able to introduce formyl, methyl and methylthio at the 4-position (forming **15a-c**) by lithiation and quench with an appropriate electrophile, followed by demethylation with hydrogen bromide.[13] There is thus a strong need to develop efficient syntheses of 4,5-disubstituted isoquinolin-1-ones with nitrogen substituents in the 5-position and opportunities for diversity at the 4-position to be able, *inter alia*, to explore the structure-activity relationships for inhibition of the numerous isoforms of PARP and other enzymes.

Chemistry

Palladium-catalysed couplings usually offer great opportunities to introduce a wide range of substituents onto a heterocyclic core under relatively mild conditions, providing chemical diversity rapidly and efficiently. Thus these methods were explored for attachment of the 4-substituents. 5-Nitroisoquinolin-1-one **16** (Scheme 2) is readily accessible as a starting material carrying the required 5-nitrogen substituent[6,9] and it was expected that a halogen could be introduced electrophilically to the 4-position, as this is the most nucleophilic in this heterocycle.[14] It proved impossible to iodinate at this position using a variety of reagents and conditions (iodine in acetic acid, N-iodosuccinimide in acetic acid, *etc*.); even activation



Scheme 1. Previous syntheses of 4,5-disubstituted isoquinolin-1-ones. *Reagents*: i, $(Ph_3P)_4Pd$, Et_3N, Bu_4NCl, DMF; ii, H_2, Pd/C, EtOH, aq. HCl; iii, Bu_3N, Ph_2O, 240°C; iv, pyridinium$^+$ Br_3^-, CH_2Cl_2; v, $(COCl)_2$, DMF, $(CH_2Cl)_2$, vi, NaOMe, MeOH; vii, BuLi, THF; viii, DMF *or* MeI *or* MeSSMe; ix, aq, HBr.

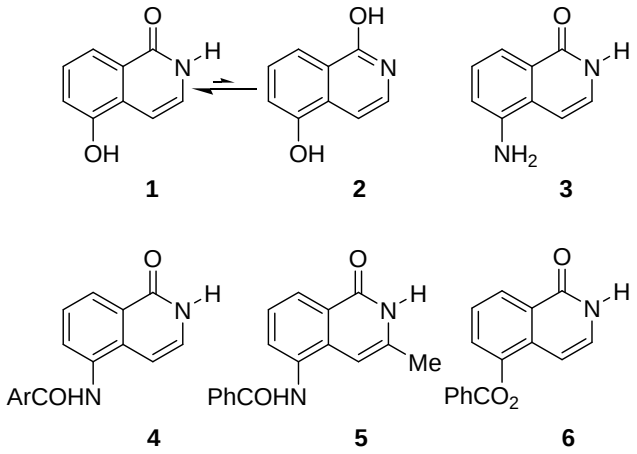
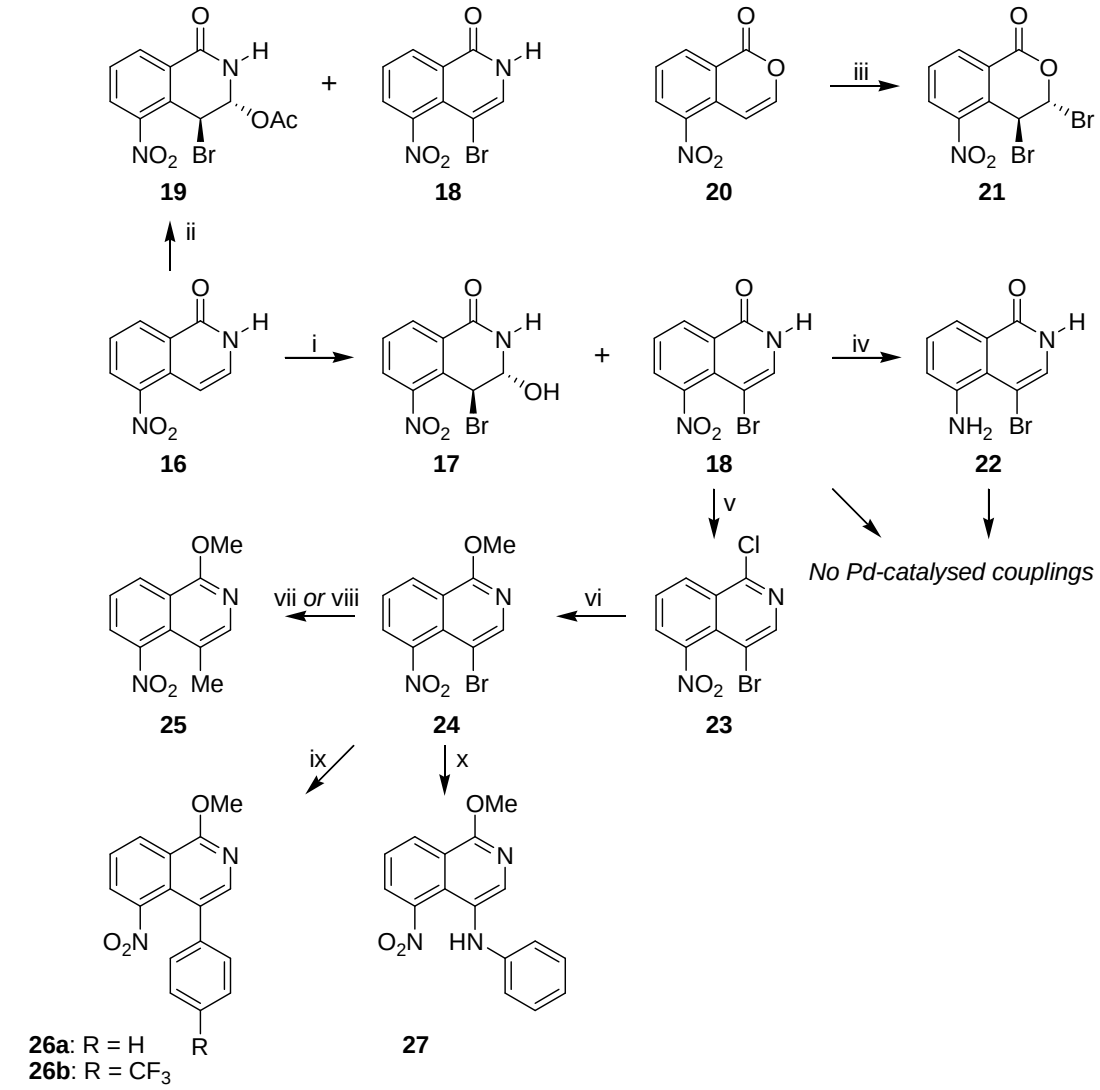


Figure 1. Structures of isoquinolin-1-one inhibitors of PARP-1 and of PARP-2. Compounds **4-6** are selective inhibitors of PARP-2.

of molecular iodine by Ag^+ ions (I_2 / AgOTf) failed to effect any conversion of the starting isoquinolin-1-one **16**. Horning *et al*.[15] have reported that reaction of 2-methyl-5-nitroisoquinolin-1-one with bromine in acetic acid afforded an equimolar mixture of 4-bromo-2-methyl-5-nitroisoquinolin-1-one and 4-bromo-3-hydroxy-2-methyl-5-nitro-3,4-dihydroisoquinolin-1-one and we sought to investigate whether or not this process could be adapted to the N-unsubstituted analogue. Pleasingly, treatment of a concentrated solution of **16** with bromine in hot acetic acid gave the required 4-bromo compound **18** in moderate yield, accompanied by a significant amount of the 3-hydroxy-3,4-dihydro analogue **17**. Heating **17** to 175°C in the absence of solvent for several hours eliminated water to give a small additional yield of **16**. Bromination of **16** with N-bromosuccinimide (NBS) in hot acetic acid in lower yield, together with a moderate yield of the 3-acetoxy-3,4-dihydro analogue **19**. Again, thermolysis of **19** (137°C) gave a small additional amount of **18** but mainly the unbrominated starting material **16**. As it is known that isocoumarins are easily converted into the corresponding isoquinolin-1-ones, bromination of 5-nitroisocoumarin **20** was also investigated; in this case, reaction with bromine in acetic acid furnished only a good yield of the *trans*-3,4-dibromo compound **21**. Interestingly, the small coupling constant (^3J = 1.7 Hz) between the 3-H and the 4-H in the ^{1}H NMR spectrum of **21** is only consistent with a *trans*-diaxial arrangement of the bromine atoms, presumably to avoid steric clash of the 3-Br with the adjacent bulky nitro group.

Unfortunately, 4-bromo-5-nitroisoquinolin-1-one **18** failed to couple in Sonogashira, Stille and Suzuki reactions under a wide variety of conditions; in most cases, **18** was recovered but with some of the debrominated analogue **16** also being obtained. The formation of **16** indicated that some palladation had taken place. To test whether or not the failure to couple was due to steric hindrance from the *peri* nitro group, **18** was reduced selectively with tin(II) chloride to the 5-amino-4-bromo compound **22**, under conditions designed to avoid hydrogenolysis of the C–Br bond.[16] Frustratingly, Pd-catalysed couplings to **22** also failed (Scheme 2).

Many isoquinolin-1-ones have very limited solubility in solvents which are appropriate for Pd-catalysed couplings and **18** and **22** are no exception. To attempt to alleviate this potential problem, the lactam moiety was masked. As shown in Scheme 2, reaction of **18** with the Vilsmeier reagent generated *in situ* from DMF and oxalyl chloride gave the 1-chloroisoquinoline **23** in excellent yield, from which the chloride was displaced by methoxide to furnish 4-bromo-1-methoxy-5-nitroisoquinoline **24**. Following Sercel's approach to **15b**, exchange of bromine for lithium with butyl lithium, followed by quench of the anion with iodomethane, furnished the 4-methyl compound **25** but in very poor yield. It could be speculated that the *peri* nitro group had interfered either with the lithiat-



Scheme 2. Pd-catalysed couplings to 4-bromo-1-methoxyisoquinolin-1-one **24**. *Reagents and conditions*: i, Br_2, AcOH, 60°C, 52% (**18**), 26% (**17**); ii, NBS, AcOH, 33% (**19**), 21% (**18**); iii, Br_2, AcOH, 52%; iv, $SnCl_2$, EtOH, 80°C, 54%; v, $(COCl)_2$, DMF, $(CH_2Cl)_2$, 80°C, 89%; vi, Na, MeOH, reflux, 82%; vii, BuLi, THF, MeI, -78°C → 20°C, 9%; viii, $SnMe_4$, $Pd_2(dba)_3$, SPhos, PhMe, 100°C, 72%; ix, $PhB(OH)_2$ *or* 4-$F_3CPhB(OH_2)$, $Pd_2(dba)_3$, SPhos, PhMe, 100°C, 86% (**28a**), 81% (**28b**); x, $PhNH_2$, $Pd_2(dba)_3$, SPhos, $KOBu^t$, 1,4-dioxane, 100°C, 45%. Compounds **17**, **19** and **21** are racemic.

ion or with the subsequent reaction with the electrophile. This lithiation / quench sequence could also not be applied to the introduction of aryl groups at the 4-positions, owing to lack of an appropriate electrophile, so other couplings were sought.

Palladium-catalysed couplings are relatively insensitive to the presence of other substituents in the substrates. Stille coupling of **24** with tetramethyltin using a conventional tetrakis(triphenylphosphine)palladium(0) catalyst failed but the $Pd_2(dba)_3$ / SPhos catalyst / ligand system introduced by Buchwald[17] for enhancing Suzuki couplings was successful in providing **25** in 72% yield. Similarly, Suzuki coupling of **24** with phenylboronic acid and with 4-trifluoromethylphenylboronic acid (an electron-poor boronic acid often associated with poor coupling yields[18]) proceeded very efficiently with $Pd_2(dba)_3$ / SPhos in toluene in providing **26a** and **26b**, respectively, in high yields. The structure of coupled product **26a** was confirmed by X-ray crystallography (Figure 2). Interestingly, rhomboids of dimensions 1 cm × 1 cm × 0.3 cm could be grown readily from ethyl acetate / hexane. The crystal structure shows clearly that the 5-nitro is rotated significantly out of the plane of the bicycle but the 4-phenyl remains coplanar with it, retaining maximum conjugation.

Exploring the generality of this reaction, Buchwald-Hartwig couplings were attempted with aniline, with thiophenol and with phenol. Only the former coupled effectively, to give the deep red 4-phenylamino analogue **27**. Optimisation of this Buchwald-Hartwig coupling of **24** with aniline revealed that the optimum ligand was SPhos (compared with XPhos, BuXPhos and JohnPhos), the optimum base was potassium *t*-butoxide (compared with tripotassium phosphate) and the optimum solvent was 1,4-dioxane (compared with toluene and DMF) (Table 1).

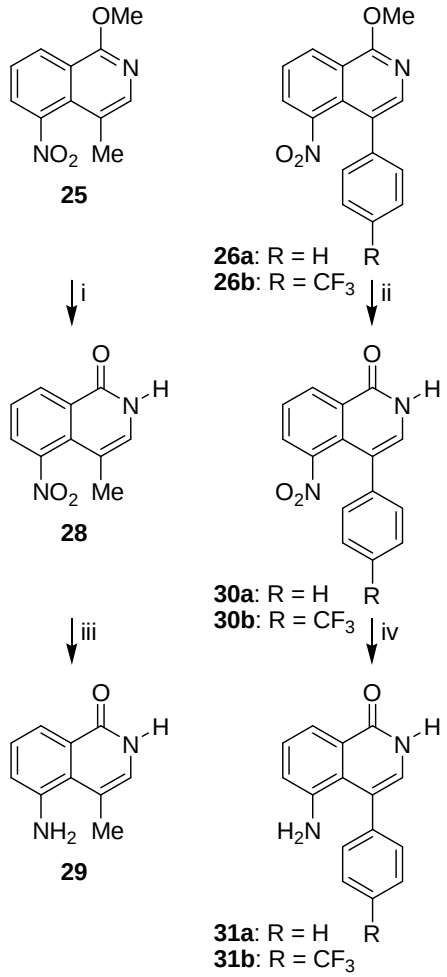
Table 1. Yields of **27** obtained during optimisation of the Buchwald-Hartwig coupling of **24** with aniline.

Ligand	PhMe / K_3PO_4	DMF / K_3PO_4	Dioxane / K_3PO_4	PhMe / KOBu^t	DMF / KOBu^t	Dioxane / KOBu^t
XPhos[a]	30%	36%	38%	32%	38%	34%
SPhos[b]	32%	38%	36%	34%	40%	45%
t-Bu-XPhos[c]	11%	5%	10%	9%	9%	14%
John-Phos[d]	0%	0%	0%	0%	0%	0%

[a] 2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl.
[b] 2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl.
[c] 2-Di-*t*-butylphosphino-2',4',6'-triisopropylbiphenyl.
[d] 2-Di-*t*-butylphosphinobiphenyl.

Scheme 3 shows how the coupled products were converted to the corresponding isoquinolin-1-ones. The lactim unit of **25** was deprotected to the lactam by demethylation with hydrobromic acid to give 4-methyl-5-nitroisoquinolin-1-one **28**. From here, simple catalytic hydrogenation of the nitro group provided the 5-amino analogue **29**. The 5-aryl-1-methoxyisoquinolines **26a,b** were similarly demethylated to the 4-aryl-5-nitroisoquinolin-1-ones **30a,b**, which were reduced to the 5-amino-4-arylisoquinolin-1-ones **31a,b**.

This demethylation is inappropriate to the synthesis of **34** and **31c** carrying a 4-(4-methoxyphenyl) group, as this substituent would also be demethylated by the hydrogen bromide. A modified sequence is shown in Scheme 4, in which the protecting 1-methoxy group is replaced by a benzyloxy group which can be removed under conditions which retain the 4-(4-methoxyphenyl) unit. Reaction of the 1-chloro compound **23** with sodium benzyloxide in boiling DMF furnished the required 1-OBn protected compound **33** in moderate yield but a significant amount of the 1-dimethylaminoisoquinoline **32** was also isolated. This material arose from thermal degradation of the solvent, liberating highly nucleophilic dimethylamine which reacted with the electrophilic **23**. The structure of **33** was confirmed by X-ray crystallography. In this structure (Figure 2), the molecule is essentially planar, with the benzyloxy function pointing away from the core. Again, the nitro group is *peri* to a large group in the 4-position (bromine), resulting in the nitro group being twisted out of plane and the C–Br bond being bent away from the nitro group. Pd-catalysed Suzuki coupling of **33** with 4-methoxyphenylboronic acid smoothly gave a high yield of **34**. From here, removal of the benzyl protecting group and reduction of the 5-nitro group was achieved in one step, giving **31c**. To provide a severe test of any steric constraints on this new Suzuki coupling to the 4-bromo-5-nitroisoquinolines, coupling of **33** with phenanthrene-9-boronic acid was attempted. Surprisingly for such a large aromatic group approaching the 4-position of the isoquinoline with the bulky *peri* 5-nitro group, coupling was effective under the standard conditions, giving a 42% yield of **35**. The X-ray crystal structure of this highly crowded extended binaphthyl was determined (Figure 2). As expected, both the 5-nitro and the 4-phenanthrene substituents are twisted severely out of the plane



Scheme 3. Formation of the 5-aminoisoquinolin-1-ones **29** and **31a,b**. *Reagents and conditions*: i, aq. HBr, 80°C, 70%; ii, aq. HBr, 50°C, 65% (**30a**), 65% (**30b**); iii, H_2, Pd/C, EtOH, aq. HCl, 65%; iv, H_2, Pd/C, EtOH, 51% (**31a**), 53% (**31b**).

of the isoquinoline to relieve the steric compression. This is shown by the asymmetry in the angles at C8 involving the phenanthrene (C7-C8-C17 126.6, C9-C8-C17 116.3°) and also by the C8-C17-C30-C29 torsion angle of 171°. Nearest neighbour 4-phenanthrene groups in the gross structure are approximately coplanar, with a separation distance of 3.68 Å.The O-benzyl group was removed by hydrogenolysis, with simultaneous reduction of the nitro group, to give **36**. This compound was insoluble in all common solvents, precluding both characterisation by NMR and any biological evaluation.

As 5-benzamidoisoquinolin-1-one **4** was highly selective for inhibition of the PARP-2 isoform,[9] **31a** was benzoylated at the exocyclic amine to furnish **37** as one example of a 4-substituted 5-benzamidoisoquinolin-1-one.

Biochemical evaluation

Selected isoquinoline-1-ones **22**, **30a**, **31b**,**c** and **37** were evaluated for their inhibition of the catalytic activities of PARP-1 and PARP-2; the data are presented in Table 2. Comparative data are also given for the non-isoform-selective inhibitor 5-AIQ **3**,[9] for **4** (Ar = Ph), which is 9.3-fold selective for inhib-

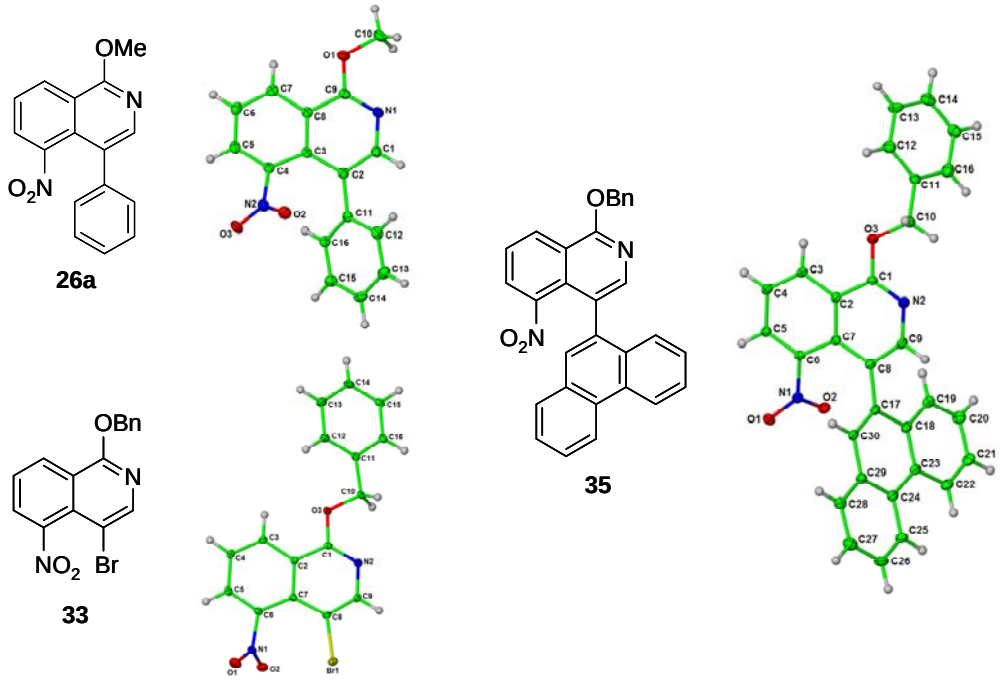
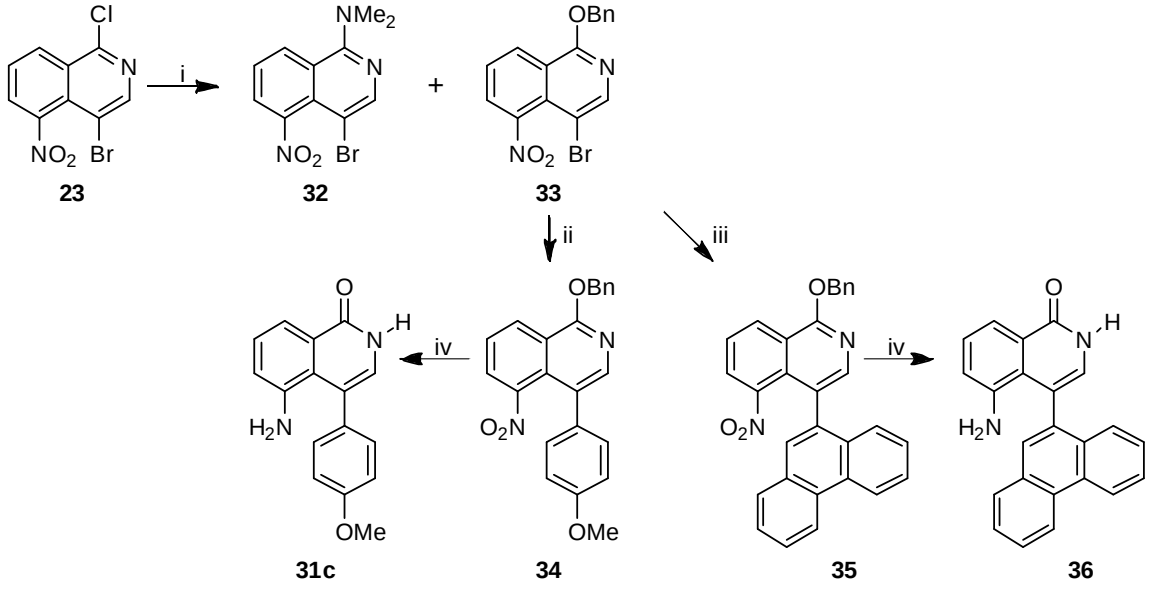
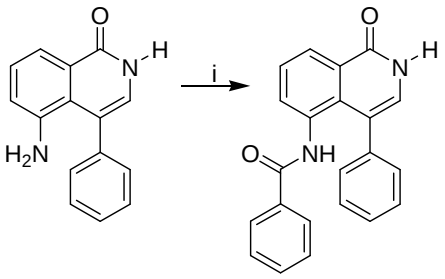


Figure 2. X-ray crystal structures of 4,5-disubstituted isoquinolines **26a**, **33** and **35**. Ellipsoids are represented at 30% probability. Solvent in the structure of **35** is omitted for clarity.



Scheme 4. Pd-catalysed couplings to 1-benzyloxy-4-bromo-5-nitroisoquinolin-1-one **33**. *Reagents and conditions*: i, BnOH, NaH, DMF, 100°C, 71% (**33**), 12% (**32**); ii, 4-MeOPhB(OH)$_2$, $Pd_2(dba)_3$, SPhos, K_3PO_4, PhMe, 100°C, 61%; iii, phenanthrene-9-boronic acid, $Pd_2(dba)_3$, SPhos, K_3PO_4, PhMe, 100°C, 42%; iv, Pd/C, H_2, EtOH, 47% (**31c**), 57% (**36**).

ition of PARP-2,[9] and for **6**, which is 2.75-fold selective for PARP-2[9] (60-fold claimed by Pellicciari *et al.*[10]). Introduction of the 4-bromo substituent in **22** led to increase in potency against both PARP isoforms, relative to the lead non-selective inhibitor 5-AIQ **3**; notably, the activity against PARP-2 was increased over 4-fold, leading to a 2.6-fold selectivity for inhibition of PARP-2 by **22**. The 5-nitro-4-phenyl analogue **30a** was non-selective and less potent than was **3**, as expected for 5-nitroisoquinolin-1-ones which are generally weaker inhibitors of PARP enzymes.[1] By contrast, **31b**, which corresponds to 5-AIQ **3** but carrying a 4-trifluorophenyl group at the 4-position, is four-times less potent than **3** against PARP-1 but more inhibitory towards PARP-2. Thus **31b** is almost as selective (7.6-fold) for PARP-2 as is most selective compound **4** (Ar = Ph; 9.3-fold) reported to date[9] and presents a new lead core to explore further the structural requirements for selec-



31a **37**

Scheme 5. Benzoylation of **31a**. *Reagents and conditions*: i, PhCOCl, pyridine, 90°C, 36%.

Table 2. Inhibition of the activities of PARP-1 and PARP-2 by 4,5-disubstituted isoquinolin-1-ones **22**, **30a**, **31b,c** and **37**; data for 5-AIQ **3**, 5-benzamidoisoquinolin-1-one **4** (Ar = Ph) and 5-benzoyloxyisoquinolin-1-one **6** are shown for comparison.[9]

Cpd. No.	4-Substituent	5-Substituent	PARP-1 IC_{50} (μM)	PARP-2 IC_{50} (μM)	Observed selectivity[a]
22	Br	H_2N	0.56	0.22	2.6
30a	Ph	O_2N	3.8	2.5	1.5
31b	4-F_3CPh	H_2N	4.1	0.54	7.6
31c	4-MeOPh	H_2N	4.7	2.2	2.2
37	Ph	PhCONH	>100	>100	-
3	H	H_2N	0.94	1.05	0.9
4 (Ar = Ph)	H	PhCONH	13.9	1.5	9.3
6	H	$PhCO_2$	4.10	1.49	2.8

[a] $IC_{50\ (PARP\text{-}1)}$ / $IC_{50\ (PARP\text{-}2)}$

tivity. Curiously, changing the electron-withdrawing trifluoromethyl group in **31b** for an electron-donating methoxy group in **31c** decreased binding to PARP-2 and hence selectivity. The most selective lead inhibitor **4** (Ar = Ph) contains a 5-benzamido group but including this into the 4-aryl series in **37** completely abolished activity against both isoforms. This is probably owing to the steric crowding between the 4- and 5-*peri*-substituents evident in the MM2-minimised structure of **37** (Figure 3), distorting the bicycle and related to that observed in the crystal structure of **35** (Figure 2). Figure 4 shows the results of *post facto* modelling of the structure of **31b** complexed to the NAD^+-binding site of human PARP-2. The starting stucture was of human PARP-2 complexed with the non-selective inhibitor ABT888.[19] PARP-2-selective inhibitor **31b** was then docked into the models using the existing bound inhibitor as template. Once docked, the inhibitor was subjected molecular mechanics and dynamics calculations to establish optimal docking conformations; during these calculations, the receptor was restrained to its original conformation. Lastly, both the inhibitors and binding pockets (radius 10Å) were subjected to molecular dynamics and finally molecular mechanics calculations to give the final structure (Figure 3).

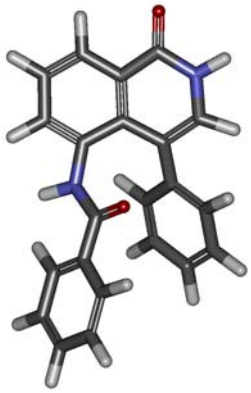


Figure 3. MM2-Minimised structure of **37**, showing steric crowding between the *peri* 4,5-substituents.

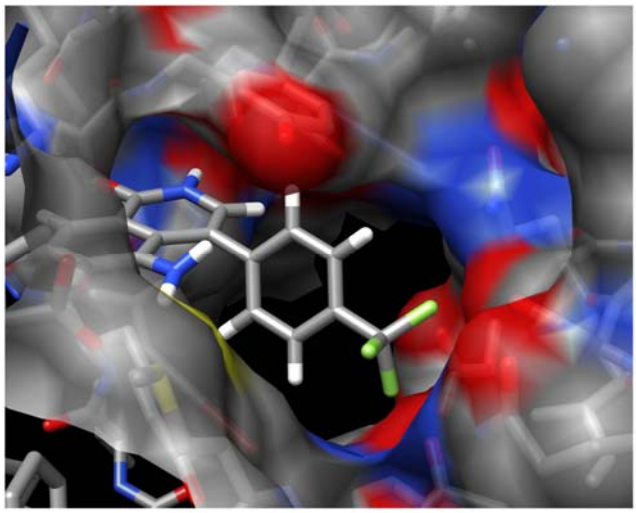


Figure 4. Molecular modelling of **31b** bound to the NAD^+-binding site of human PARP-2.

The larger binding pocket of PARP-2[9] accomodates the 4-(4-trifluoromethyphenyl) group, whereas the smaller pocket of PARP-1[9] does not.

Conclusions

In this paper, we report that palladium-catalysed couplings (Stille, Suzuki, Buchwald-Hartwig) to the sterically very crowded 4-position of 1-alkoxy-4-bromo-5-nitroisoquinolines **24** and **33** are very efficient in providing 4-alkyl- and 4-aryl-1-alkoxy-5-nitroisoquinolines. The Suzuki coupling with arylboronic acids is insensitive to electron-withdrawing (-CF_3) and electron-donating (-OMe) groups on the phenylboronic acid. Surprisingly, major steric bulk is also tolerated in the coupling reaction, in that phenanthrene-9-boronic acid is also a satisfactory coupling partner in the formation of **35.** Analogous couplings were not possible using the corresponding isoquinolin-1-ones **18** and **22**, probably owing to poor solubility of these lactams. 1-Alkoxyisoquinolines can be considered as masked isoquinoline-1-ones[22] and **25**, **26a,b**, **34** and **35** are no exception. Demethylation of the 1-methoxy compounds **25** and **26a,b** led to the 5-nitroisoquinolin-1-ones **28** and **30a,b**,

respectively, for later reduction to the 4-substituted 5-AIQs **29** and **31a,b**. Simplifying the syntheses of 4-substituted 5-AIQs further, catalytic hydrogenolysis simultaneously removed the protecting O-benzyl group and reduced the nitro function of **34** and **35** to access the 4-aryl 5-AIQs **31c** and **36**, respectively. Benzoylation of **31a** gave 5-benzamido-4-phenylisoquinolin-1-one **37**, despite the severe crowding in the product. Selected 4,5-disubstituted isoquinoline-1-ones were evaluated as isoform-selective inhibitors of PARP-2; 4-(4-trifluoromethylphenyl)-5-AIQ **31b** was particularly potent and selective and is a new lead in the search for isoform-selectivity for this important family of enzymes.

Experimental

General

NMR spectra were recorded on JEOL Delta 270 and Varian Mercury 400 spectrometers of solutions in deuteriochloroform, unless otherwise stated; coupling constants (*J*) are given in Hz. Mass spectra were obtained using VG7070E and Bruker microTOF™ spectrometers in the ES^+ mode. IR spectra were measured on a Perkin-Elmer RXI FTIR spectrometer as KBr discs. The stationary phase for chromatography was silica gel. All reactions were carried out at ambient temperature, unless otherwise stated. Solvents were evaporated under reduced pressure. Melting points were determined using a Reichert-Jung Thermo Galen instrument and are uncorrected. SPhos refers to 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl and Pd_2dba_3 refers to tris(dibenzylideneacetone)dipalladium.

4-Bromo-5-nitroisoquinolin-1-one (18) and 4-bromo-3-hydroxy-5-nitro-3,4-dihydroisoquinolin-1-one (17)

Bromine (5.0 g, 32 mmol) in acetic acid (5.0 mL) was added slowly to a suspension of **16** (6.0 g, 32 mmol) in acetic acid (15 mL). The mixture was stirred at 60°C for 16 h, then cooled and poured onto ice-water (60 mL). The precipitate was collected, washed (methanol) and dried. Chromatography (hexane / ethyl acetate 6:1) gave **18** (4.5 g, 52%) as a pale orange solid: mp 229-232°C; ν_{max} 3467, 1674, 1534, 1368 cm^{-1}; δ_H ($(CD_3)_2CO$) 7.73 (1 H, s, 3-H), 7.77 (1 H, t, *J* 7.8, 7-H), 8.10 (1 H, dd, *J* 7.8, 1.6, 6-H), 8.61 (1 H, dd, *J* 8.6, 1.9, 8-H); δ_C ($(CD_3)_2CO$) (HMQC / HMBC) 90.4 (4-C), 128.0 (10-C), 128.3 (7-C), 129.5 (6-C), 129.9 (9-C), 132.1 (8-C), 135.6 (3-C), 147.3 (5-C), 159.0 (1-C); *m/z* 292.9354 (M + Na) ($C_9H_5{}^{81}BrN_2O_3Na$ requires 292.9312), 290.9376 (M + Na) ($C_9H_5{}^{79}BrN_2O_3Na$ requires 290.9332), 267.9478 (M) ($C_9H_5{}^{79}BrN_2O_3$ requires 267.9484); Found: C, 40.60; H, 1.61; N, 10.19. Calc. for $C_9H_5BrN_2O_3$: C, 40.18; H, 1.87; N, 10.41%. Further elution gave **17** (2.4 g, 26%) as a pale yellow solid: mp 170-172°C; δ_H ($(CD_3)_2CO$) 5.52 (1 H, dd, *J* 5.7, 1.2, 3-H), 6.73 (1 H, d, *J* 5.9, 4-H), 7.82 (1 H, t, *J* 7.9, 7-H), 8.00 (1 H, dd, *J* 7.9, 1.5, 6-H), 8.30 (1 H, dd, *J* 7.7, 1.5, 8-H), 8.55 (1 H, br, NH).

4-Bromo-5-nitroisoquinolin-1-one (18) and 3-acetoxy-4-bromo-5-nitro-3,4-dihydroisoquinolin-1-one (19)

N-Bromosuccinimide (90 mg, 0.5 mmol) was stirred with **16** (100 mg, 0.5 mmol) in acetic acid (5 mL) for 30 min. The mixture was poured into ice-H_2O (100 mL) and stirred for 10 min. Extraction (ethyl acetate), washing (aq. sodium hydrogen carbonate, water), drying, evaporation and chromatography (hexane / ethyl acetate 4:1) yielded **19** (70 mg, 33%) as a pale buff solid: mp 137°C; ν_{max} 3462, 1675, 1534, 1335 cm^{-1}; δ_H ($(CD_3)_2CO$) 2.08 (3 H, s, Me), 5.79 (1 H, d, *J* 4.7, 4-H), 5.95 (1 H, m, 3-H), 7.82 (1 H, t, *J* 7.9, 7-H), 8.33 (1 H, dd, *J* 8.2, 1.5, 6-H), 8.45 (1 H, dd, *J* 8.0, 1.2, 8-H). Further elution gave **18** (300 mg, 21%), with data as above.

(±)-*trans*-3,4-Dibromo-5-nitroisocoumarin (21)

Bromine (210 mg, 1.3 mmol) in acetic acid (1.0 mL) was added to 5-nitroisocoumarin **20**[6] (250 mg, 1.3 mmol) in acetic acid (2.5 mL) and the mixture was stirred for 2 h before being poured into ice-H_2O (100 mL). The suspension was stirred for 10 min, then extracted (CH_2Cl_2). Drying, evaporation and chromatography (hexane / EtOAc 4:1) yielded **21** (240 mg, 52%) as white crystals: mp 116-118°C; ν_{max} 1761, 1528, 1346 cm^{-1}; δ_H 6.32 (1 H, d, *J* 1.7, 4-H), 6.90 (1 H, d, *J* 1.7, 3-H), 7.80 (1 H, t, *J* 8.2, 7-H), 8.52 (1 H, s, 6-H), 8.64 (1 H, s, 8-H); δ_C (HMQC / HMBC) 38.4 (4-C), 78.2 (3-C), 125.1 (9-C), 130.9 (7-C), 131.3 (8-C), 133.0 (10-C), 135.7 (6-C), 145.3 (5-C), 158.3 (1-C); *m/z* 373.8455 (M + Na) ($C_9H_6{}^{79}Br^{81}BrNO_4Na$ requires 373.8463), 353.8621 (M + H) ($C_9H_6{}^{81}Br_2NO_4$ requires 353.8623), 351.8647 (M + H) ($C_9H_6{}^{79}Br^{81}BrNO_4$ requires 351.8642), 349.8658 (M + H) ($C_9H_6{}^{79}Br_2NO_4$ requires 349.8664).

5-Amino-4-bromoisoquinolin-1(2*H*)-one (22)

Compound **18** (540 mg, 2.0 mmol) was heated with tin(II) chloride (1.21 g, 6.4 mmol) in ethanol (20 mL) at 80°C for 4 h, then carefully poured into ice-H_2O (75 mL). The suspension was made alkaline with aq. NaOH and the precipitate was filtered. Extraction of the filtrate (EtOAc), evaporation and chromatography (ethyl acetate / hexane 4:1) gave **22** (250 mg, 54%) as a pale buff powder: mp 210-212°C; ν_{max} 3443, 3321, 1661, 1624 cm^{-1}; δ_H ($(CD_3)_2SO$) 5.92 (2 H, br s, NH_2), 7.73 (1 H, s, 3-H), 7.02 (1 H, dd, *J* 8.2, 1.6, 6-H), 7.22 (1 H, s, 3-H), 7.25 (1 H, t, *J* 8.2, 7-H), 7.54 (1 H, dd, *J* 7.8, 1.2, 8-H), 11.34 (1 H, br s, NH); δ_C ($(CD_3)_2SO$) (HMQC / HMBC) 93.10 (4-C), 115.83 (8-C), 119.17 (6-C), 119.42 (10-C), 128.04 (3-C), 128.44 (9-C), 128.56 (7-C), 144.72 (5-C), 160.83 (1-C); *m/z* 238.9815 (M + H) ($C_9H_8{}^{79}BrN_2O$ requires 238.9820).

4-Bromo-1-chloro-5-nitroisoquinoline (23)

Oxalyl chloride (5.3 mL, 7.67 g, 60.4 mmol) was added dropwise during 30 min to dry dimethylformamide (4.7 mL, 4.4 g, 60.4 mmol) in 1,2-dichloroethane (35 mL) at 0°C. The suspension was stirred at room temperature for 10 min, then **18** (7.3 g, 27 mmol) was added. The mixture was then heated at 80°C for 6 h, allowed to cool and diluted with CH_2Cl_2. Washing (water), drying and evaporation gave **23** (7.0 g, 89%) as a yellow solid: mp 164-166°C; δ_H 7.82 (1 H, t, *J* 7.6, 7-H), 8.01 (1 H, dd, *J* 7.6, 1.2, 6-H), 8.62 (1 H, s, 3-H), 8.65 (1 H, dd, *J* 7.6, 1.2, 8-H); δ_C 112.4, 127.1, 127.8, 128.2, 128.6, 131.0, 147.4, 147.6, 152.0.

4-Bromo-1-methoxy-5-nitroisoquinoline (24)

Finely divided sodium (700 mg, 31 mmol) was added to **23** (5.0 g, 17 mmol) in dry methanol (90 mL) and the mixture was boiled under reflux for 16 h. The solvent was then evaporated until 20 mL remained; the residue was diluted with H_2O and extracted (chloroform). Drying and evaporation gave **24** (4.0 g, 82%) as a yellow solid: mp 154-157°C; δ_H 4.18 (3 H, s, Me), 7.63 (1 H, t, *J* 7.8, 7-H), 7.88 (1 H, dd, *J* 7.8, 1.1, 6-H), 8.29 (1 H, s, 3-H), 8.48 (1 H, dd, *J* 7.8, 1.1, 8-H); δ_C 54.6, 104.2, 110.0, 121.9, 126.3, 126.9, 128.5, 146.2, 147.0, 160.3; Found: C, 42.43; H, 2.63; N, 9.69. Calc. For $C_{10}H_7BrN_2O_3$: C, 42.43; H, 2.49; N, 9.90%.

1-Methoxy-4-methyl-5-nitroisoquinoline (25) Method A

Butyllithium in tetrahydrofuran (1.6 M, 0.24 mL, 0.38 mmol) was added to **24** (100 mg, 0.35 mmol) in dry tetrahydrofuran (9 mL) at -78°C. The suspension was stirred for 20 min. Iodomethane (55.4 mg, 0.39 mmol) in tetrahydrofuran (1 mL) was added and the mixture allowed to warm to 20°C during 1 h. The reaction was quenched with water. Extraction (dichloromethane), evaporation and chromatography (hexane / ethyl acetate 15:1) gave **25** (7 mg, 9%) as a yellow-orange solid: mp 90-93°C; δ_H 2.91 (3 H, s, 4-Me), 4.09 (3 H, s, OMe), 7.50 (1 H, t, *J* 8.6, 7-H), 7.75 (1 H, d, *J* 7.4, 8-H), 7.87 (1 H, s, 3-H) 8.42 (1 H, d, *J* 7.4, 6-H); δ_C (HMBC / HMQC) 16.00 (4-Me), 53.94 (OMe), 120.00 (4-C), 125.02 (7-C), 125.34 (4a-C), 125.66 (8-C), 128.34 (6-C), 128.91 (8a-C), 143.24 (5-C), 143.58 (3-C), 159.99 (1-C); *m/z* 241.0582 (M + Na) ($C_{10}H_{10}N_2NaO_3$ requires 241.0589), 219.0772 (M + H) ($C_{10}H_{11}N_2O_3$ requires 219.0770).

1-Methoxy-4-methyl-5-nitroisoquinoline (25) Method B

Compound **24** (1.00 g, 3.52 mmol), $Pd_2(dba)_3$ (180 mg, 0.35 mmol), SPhos (140 mg, 0.70 mmol) and tetramethyltin (0.95 g, 5.28 mol) were placed in a dry flask. Degassed toluene (20 mL) was added and the mixture was stirred at 70°C for 7 d. Evaporation and chromatography (hexane / ethyl acetate 15:1) gave **25** (0.77 g, 72%) as a yellow-orange solid with data as above.

1-Methoxy-5-nitro-4-phenylisoquinoline (26a)

Compound **24** (1.00 g, 3.53 mmol), $Pd_2(dba)_3$ (180 mg, 0.35 mmol), SPhos (140 mg, 0.70 mmol), tripotassium phosphate (1.5 g, 7.06 mmol) and phenylboronic acid (640 mg, 5.30 mmol) were placed in a dry flask. Degassed toluene (40 mL) was added and the mixture was stirred at 100°C for 16 h. Evaporation and chromatography (hexane / ethyl acetate 10:1) gave **26a** (850 mg, 86%) as yellow crystals: mp 118-120°C; δ_H 4.20 (3 H, s, OMe), 7.27-7.31 (2 H, m, Ph 2,6-H_2), 7.38-7.43 (3 H, m, Ph 3,4,5-H_3), 7.62 (1 H, t, *J* 8.0, 7-H), 7.97 (1 H, dd, *J* 8.0, 1.2, 6-H or 8-H), 8.06 (1 H, s, 3-H), 8.60 (1 H, dd, *J* 8.0, 1.2, 8-H or 6-H); δ_C 54.4, 120.7, 124.5, 125.5, 127.4, 127.7, 127.8, 128.1, 128.4, 129.1, 137.5, 144.7, 147.6, 160.3; *m/z* 303.0740 (M + Na) ($C_{16}H_{11}NaN_2O_3$ requires 303.0746); 281.0915 (M + H) ($C_{16}H_{12}N_2O_3$ requires 281.0926); Found: C, 68.50; H, 4.26; N, 10.19. Calc. for $C_{16}H_{12}N_2O_3$: C, 68.57; H, 4.32; N, 10.00%.

1-Methoxy-5-nitro-4-(4-trifluoromethylphenyl)isoquinoline (26b)

Compound **24** was treated with 4-trifluoromethylphenylboronic acid, $Pd_2(dba)_3$, SPhos and K_3PO_4 in toluene, as for the synthesis of **26a**, to give **26b** (81%) as yellow crystals: mp 95-97°C; δ_H 4.25 (3 H, s, OMe), 7.43 (2 H, d, *J* 8.8, Ar 2,6-H_2), 7.68-7.72 (3 H, m, 7-H and Ar 3,5-H_3), 8.05 (1 H, dd, *J* 8.4, 1.2, 6-H), 8.07 (1 H, s, 3-H), 8.66 (1 H, dd, *J* 8.4, 1.2, 8-H); δ_C 54.5, 120.7, 123.1, 125.4 (q, *J* 3.7, Ar 3.5-C_2), 125.8, 127.3 (m, CF_3), 127.6, 128.3, 129.4, 130.0 (m, Ar 4-C), 141.2, 145.0, 160.8; *m/z* 371.0631 (M + Na) ($C_{17}H_{11}F_3NaN_2O_3$ requires 371.0619), 349.0805 (M + H) ($C_{17}H_{12}F_3N_2O_3$ requires 349.0800); Found: C, 58.47; H, 3.23; N, 7.96. Calc. for $C_{17}H_{11}F_3N_2O_3$: C, 58.63; H, 3.18; N, 8.05%.

1-Methoxy-5-nitro-4-phenylaminoisoquinoline (27)

Compound **24** (1.00 g, 3.5 mmol), $Pd_2(dba)_3$ (180 mg, 0.35 mmol), SPhos (140 mg, 0.70 mmol), potassium *t*-butoxide (790 mg, 7.06 mmol) and aniline (0.49 g, 5.3 mmol) were placed in a dry flask. Degassed 1,4-dioxane (40 mL) was added and the mixture was stirred at 100°C for 16 h. Evaporation and chromatography (hexane / ethyl acetate 10:1) gave **27** (470 mg, 45%) as a deep red solid: mp 124-126°C; δ_H 4.17 (3 H, s, OMe), 5.56 (1 H, s, NH), 6.61 (2 H, dd, *J* 7.4, 1.1, Ph 2,6-H_2), 6.79 (1 H, t, *J* 7.4, Ph 4-H), 7.14 (2 H, t, *J* 7.4, Ph 3,5-H_2), 7.59 (1 H, d, *J* 8.2, 7-H), 7.80 (1 H, dt, *J* 8.2, 1.2, 8-H), 8.14 (1 H, d, *J* 1.1, 3-H), 8.51 (1 H, dd, *J* 8.2, 1.2, 6-H); δ_C 54.3, 114.2, 119.5, 121.2, 124.7, 125.8, 126.3, 127.8, 128.7, 129.3, 142.3, 146.8, 158.7; *m/z* 318.0850 (M + Na) ($C_{16}H_{13}N_3NaO_3$ requires 318.0855), 296.1027 (M + H) ($C_{16}H_{14}N_3O_3$ requires 296.1035.

4-Methyl-5-nitroisoquinolin-1-one (28)

Compound **25** (200 mg, 0.92 mmol) was stirred in aq. hydrobromic acid (48%, 30 mL) at 80°C for 4 h. Evaporation and recrystallisation (hexane / ethyl acetate) gave **28** (131 mg, 70%) as a pale buff solid mp: mp 211-214°C (lit.[11] mp 209-211°C); δ_H ($(CD_3)_2CO$) 2.02 (3 H, s, Me), 7.22 (1 H, d, *J* 5.1, 3-H), 7.66 (1 H, t, *J* 7.8, 7-H), 8.13 (1 H, dd, *J* 7.8, 1.3, 6-H), 8.50 (1 H, dd, *J* 7.8, 1.3, 8-H), 11.64 (1 H, br, NH).

5-Amino-4-methylisoquinolin-1-one hydrochloride (29)

Compound **28** (116 mg, 0.56 mmol) was stirred with palladium on charcoal (10%, 100 mg) in ethanol (14 mL) and aq. hydrochloric acid (34%, 0.4 mL) under hydrogen for 2 h. The suspension was filtered through Celite®. The Celite® pad and residue were suspended in water (100 mL) and heated. The hot suspension was filtered through a second Celite® pad. Evaporation of the solvent and drying gave **29** (78 mg, 65%) as a pale buff solid: mp 225-228°C (lit.[11] mp 227-229°C); δ_H (D_2O) 2.37 (3 H, s, Me), 6.94 (1 H, s, 3-H), 7.42 (1 H, t, *J* = 8.2, 7-H), 7.63 (1 H, d, *J* = 7.8, 6-H), 8.14 (1 H, d, *J* = 8.2, 8-H).

5-Nitro-4-phenylisoquinolin-1-one (30a)

Compound **26a** was treated with aq. hydrobromic acid, as for the synthesis of **28**, to give **30a** (65%) as yellow crystals: mp 211-214°C; δ_H ($(CD_3)_2SO$) 7.20 (3 H, m, 3-H and Ph 2,6-H_2),

7.32 (3 H, m, Ph 3,4,5-H_3), 7.70 (1 H, t, *J* 7.6, 7-H), 8.14 (1 H, dd, *J* 7.8, 1.2, 6-H), 8.58 (1 H, dd, *J* 7.8, 1.2, 8-H); δ_C ($(CD_3)_2SO$) 113.5, 126.4, 127.2, 127.6, 128.0, 128.2, 128.5, 129.0, 131.6, 133.1, 136.7, 147.0, 159.7; *m/z* 289.0598 (M + Na) ($C_{15}H_{10}NaN_2O_3$ requires 289.0589); 267.0761 (M + H) ($C_{15}H_{11}N_2O_3$ requires 267.0770); Found: C, 68.60; H, 3.48; N, 10.49. Calc. for $C_{15}H_{10}N_2O_3$: C, 67.67; H, 3.59; N, 10.52%.

5-Nitro-4-(4-trifluoromethylphenyl)isoquinolin-1-one (30b)

Compound **23b** was treated with aq. hydrobromic acid, as for the synthesis of **28**, to give **30b** (65%) as yellow crystals: mp 283-285°C; δ_H ($(CD_3)_2SO$) 7.34 (1 H, d, *J* 6.5, 3-H), 7.46 (2 H, d, *J* 7.8, Ar 3,5-H_2), 7.70 (2 H, d, *J* 7.8, Ar 2,6-H_2), 7.73 (1 H, t, *J* 8.2, 7-H), 8.22 (1 H, dd, *J* 8.2, 1.2, 6-H), 8.61 (1 H, dd, *J* 8.2, 1.2, 8-H), 12.09 (1 H, d, *J ca*. 5.5, NH); δ_C ($(CD_3)_2SO$) 112.0, 125.1 (q, *J* 3.8, Ar 3,5-C_2), 126.7, 127.7, 128.0, 128.1, 128.2, 129.3, 131.9 (m, Ph C-4), 134.0 (m, CF_3), 141.1, 146.7, 159.8; Found: C, 57.14; H, 2.67; N, 8.04. Calc. for $C_{16}H_9F_3N_2O_3$: C, 57.50; H, 2.71; N, 8.38%.

5-Amino-4-phenylisoquinolin-1-one (31a)

Compound **30a** (46 mg, 0.17 mmol) was stirred with palladium on charcoal (10%, 50 mg) in ethanol (15 mL) under hydrogen for 6 h. The suspension was then filtered through Celite®. Evaporation of the solvent and drying gave **31a** (21 mg, 51%) as a pale yellow solid: mp 236-240°C; δ_H ($(CD_3)_2SO$) 4.45 (2 H, s, NH_2), 6.70 (1 H, brs, 3-H), 6.86 (1 H, dd, *J* 7.8, 1.2, 6-H), 7.23 (1 H, t, *J* = 7.8, 7-H), 7.36 (2 H, dd, *J* 7.3, 1.2, Ph 2,6-H_2), 7.41-7.47 (3 H, m, Ph 3,4,5-H_3), 7.60 (1 H, d, *J* 7.8, 1.2, 8-H), 11.20 (1 H, br, NH); δ_C ($(CD_3)_2SO$) (HMBC / HMQC) 116.2 (8-C), 118.40 (6-C), 122.6 (4a-C) 125.4 (4-C), 127.4 (3-C), 128.0 (7-C), 128.3 (Ph 4-C), 129.1 (Ph 3,5-C_2), 130.2 (8a-C), 130.3 (Ph 2,6-C_2), 141.1 (Ph 1-C), 142.3 (5a-C), 160.1 (1-C); *m/z* 259.0841 (M + Na) ($C_{15}H_{11}NaN_2O$ requires 259.0847), 237.1017 (M + H) ($C_{15}H_{12}N_2O$ requires 237.1022); Found: C, 76.68; H, 5.46; N, 11.43. Calc. for $C_{15}H_{12}N_2O$: C, 76.26; H, 5.12; N, 11.37%.

5-Amino-4-(4-trifluoromethylphenyl)isoquinolin-1-one (31b)

Compound **30b** was treated with hydrogen and palladium on charcoal, as for the synthesis of **31a**, to give **31b** (53%) as a pale yellow solid: mp 265-267°C; δ_H ($(CD_3)_2SO$) 4.37 (2 H, s, NH_2), 6.81 (1 H, brs, 3-H), 6.93 (1 H, dd, *J* 7.8, 1.2, 6-H), 7.27 (1 H, t, *J* 7.8, 7-H), 7.57 (2 H, d, *J* 7.6, Ar 2,6-H_2), 7.63 (1 H, dd, *J* 7.8, 1.2, 6-H), 7.77 (1 H, d, *J* 7.6, 8-H), 11.31 (1 H, brs, NH); δ_C ($(CD_3)_2SO$) 112.9, 114.7, 115.8, 118.2, 120.9, 124.9 (m, Ph 3,5-C_2), 127.5, 127.8, 127.9 (m, CF_3 or Ar C-4), 128.8 (m, Ar C-4 or CF_3), 130.2, 130.5, 143.0, 144.2, 161.5; *m/z* 327.0729 (M + Na) ($C_{17}H_{11}F_3NaN_2O$ requires 327.0712), 305.0905 (M + H) ($C_{17}H_{12}F_3N_2O$ requires 305.0902); Found: C, 63.21; H, 3.69; N, 9.30. Calc. for $C_{17}H_{11}F_3N_2O$: C, 63.16; H, 3.64; N, 9.21%.

5-Amino-4-(4-methoxyphenyl)isoquinolin-1-one (31c)

Compound **34** was treated with hydrogen and palladium on charcoal, as for the synthesis of **31a**, to give **31c** (32 mg, 47%) as a pale buff solid: mp 240-243°C; δ_H ($(CD_3)_2SO$) 4.03 (3 H, s, Me), 4.62 (2 H, br s, NH_2), 6.85 (1 H, dd, *J* 7.8, 1.2, 6-H), 6.93 (2 H, d, *J* 8.6, Ar 3,5-H_2), 7.34 (2 H, d, *J* = 8.6, Ar 2,6-H_2), 7.39 (1 H, d, *J* 8.6, Ar 3.5-H_2), 7.46 (1 H, t, *J* 7.8, 7-H), 7.60 (1 H, d, *J* 7.8, 1.2, 8-H), 10.86 (1 H, br, NH); δ_C ($(CD_3)_2SO$) 55.4, 112.1, 112.7, 113.9, 114.3, 115.0, 122.4, 126.8, 127.4, 127.5, 130.5, 143.4, 159.0, 161.4; *m/z* 267.1105 (M + H) ($C_{16}H_{15}N_2O_2$ requires 267.1134); Found: C, 76.68; H, 5.46; N, 11.43. Calc. for $C_{16}H_{14}N_2O_2$: C, 76.26; H, 5.12; N, 11.37%.

1-Benzyloxy-4-bromo-5-nitroisoquinoline (33) and 4-bromo-1-dimethylamino-5-nitroisoquinoline (32)

Benzyl alcohol (680 mg, 6.3 mmol) was added to sodium hydride (300 mg, 12.5 mmol) in dry dimethylformamide (10 mL) and the mixture was stirred for 30 min. Compound **20** (1.5 g, 5.2 mmol) in dry dimethylformamide (30 mL) was added and the suspension was heated at 100°C for 48 h. The solvent was evaporated until 5 mL remained. The residue was diluted with water and extracted (chloroform). Evaporation and chromatography (hexane / ethyl acetate 15:1) gave **33** (1.3 g, 71%) as a yellow solid: mp 106-108°C; δ_H 5.58 (2 H, s, CH_2), 7.40 (3 H, m, Ph 3,4,5-H_3), 7.52 (2 H, d, *J* 7.1, Ph 2,6-H_2), 7.62 (1 H, t, *J* 7.8, 7-H), 7.90 (1 H, dd, *J* 7.1, 1.2, 8-H), 8.33 (1 H, s, 3-H), 8.55 (1 H, dd, *J* 7.1, 1.2, 6-H); δ_C 68.96 (CH_2), 104.5, 122.0, 126.3, 126.9, 127.0, 128.2, 128.3, 128.5, 128.6, 136.1, 146.2, 147.1, 159.7; *m/z* 382.9826 (M + Na) ($C_{16}H_{11}{}^{81}BrNaN_2O_3$ requires 382.9830); 380.9860 (M + Na) ($C_{16}H_{11}{}^{79}BrNaN_2O_3$ requires 380.9851), 359.0039 (M + H) ($C_{16}H_{12}{}^{79}BrN_2O_3$ requires 359.0031). Further elution gave **32** (184 mg, 12%) as a red-orange solid mp 127-130°C; δ_H 3.14 (6 H, s, $NMe)_2$, 7.52 (1 H, t, *J* = 7.6, 7-H), 7.88 (1 H, dd, *J* = 7.6, 1.2, 6-H), 8.26 (1 H, dd, *J* = 7.6, 1.2, 8-H), 8.30 (1 H, s, 3-H); δ_C (HMQC / HMBC) 43.06 (NMe_2), 103.28 (4-C), 122.78 (8a-C), 124.21 (4-C), 126.38 (6-C), 127.95 (4a-C), 130.65 (8-C), 147.04 (3-C), 147.42 (5-C), 160.87 (1-C); *m/z* 319.9840 (M + Na) ($C_{11}H_{10}{}^{81}BrNaN_3O_2$ requires 319.9834), 317.9848 (M + Na) ($C_{11}H_{10}{}^{79}BrNaN_3O_2$ requires 317.9854).

1-Benzyloxy-4-(4-methoxyphenyl)-5-nitroisoquinoline (34)

Compound **33** (500 mg, 1.4 mmol), $Pd_2(dba)_3$ (72 mg, 0.14 mmol), SPhos (168 mg, 0.70 mmol), tripotassium phosphate (594 mg, 2.8 mmol) and 4-methoxyphenylboronic acid (317 mg, 2.1 mmol) were placed in a dry flask. Degassed toluene (20 mL) was added and the mixture was stirred at 100°C for 16 h. Chromatography (hexane / ethyl acetate, 20:1) gave **34** (330 mg, 61%) as a yellow solid: mp 162-164°C; δ_H 3.85 (3 H, s, OMe), 5.64 (2 H, s, CH_2), 6.93 (2 H, d, *J* 8.6, Ar 3,5-H_2), 7.21 (2 H, d, *J* 8.6, Ar 2,6-H_2), 7.36-7.45 (3 H, m, Ph 3,4,5-H_3), 7.55 (2 H, d, *J* 7.5, Ph 2,6-H_2), 7.60 (1 H, t, *J* 8.2, 7-H), 7.95 (1 H, d, *J* 7.4, 6-H or 8-H), 8.04 (1 H, s, 3-H), 8.63 (1 H, d, *J* 7.4, 8-H or 6-H); δ_C (HMBC / HMQC) δ 55.2 (Me), 68.6 (CH_2), 108.7, 113.8 (Ar 3,5-C_2), 120.7 (8a-C), 124.4 (Ar 1-C), 125.4 (7-C), 127.2 (6-C), 128.0 (Ph 4-C), 128.1 (4a-C), 128.2 (Ph 3.5-C_2), 128.6 (Ph 2,6-C_2), 129.0 (8-C), 129.3 (Ar 2,6-C_2), 129.8 (4-C), 131.5 (Ar 4-C), 136.7 (Ph 1-C), 147.7 (3-C), 159.2 (5-C), 159.5 (1-C); *m/z* 409.1164 (M + Na) ($C_{23}H_{17}N_2NaO_4$ requires 409.1164), 387.1366 (M + H) ($C_{23}H_{19}N_2O_4$ requires 387.1345); Anal. Found: C, 71.56; H, 4.82; N, 7.31. Calc. for $C_{23}H_{18}N_2O_4$: C, 71.49; H, 4.70; N,

7.25%.

1-(Benzyloxy)-5-nitro-4-(phenanthren-9-yl)isoquinoline (35)

Compound **33** was treated with $Pd_2(dba)_3$, SPhos, tripotassium phosphate and phenanthrene-9-boronic acid, as for the synthesis of **34**, to give **35** (42%) as yellow crystals: mp 102-105°C; δ_H 5.72 (2 H, s, CH_2), 7.41 (1 H, t, *J* 7.8, Ph 4-H), 7.47 (2 H, t, *J* 7.8, Ph 3,5-H_2), 7.52 (1 H, td, *J* 7.8, 1.2, Phen 3 or 6-H), 7.56 (1 H, s, Phen 9-H), 7.60-7.65 (3 H, m, Ph 2,6-H_2 and 7-H), 7.66-7.71 (2 H, m, Phen 1,8-H_2), 7.82 (2 H, t, *J* 7.4, Phen 2,7-H_2), 7.85 (1 H, dd, *J* 7.6, 1.2, 6-H), 8.71 (1 H, dd, *J* 7.6, 1.2, 8-H), 8.74 (1 H, d, *J* 7.8, Phen 4-H or 5-H), 8.79 (1 H, d, *J* 7.8, Phen 5-H or 4-H); δ_C (HMQC / HMBC) 68.8 (CH_2), 120.8, 122.1 (Phen 1-C), 122.7, 123.1, 125.6, 126.4 (Phen 2-C or 7-C), 126.6, 126.8, 127.0 (8-C), 127.0, 128.2 (Ph 2,6-C_2), 128.3 (Ph 4-C), 128.4, 128.7, 128.8 (Ph 3,5-C_2), 129.0 (Phen 7-C or 2-C), 129.2, 130.3, 130.6, 131.0, 131.4, 132.7, 136.7 (Ph 1-C), 145.8 (3-C), 147.7 (5-C), 160.0 (1-C); *m/z* 479.1360 (M + Na) ($C_{30}H_{20}N_2NaO_3$ requires 479.1372), 457.1571 (M + H) ($C_{30}H_{21}N_2O_3$ requires 457.1552).

5-Amino-4-(phenanthren-9-yl)isoquinolin-1-one (36)

Compound **35** was treated with hydrogen and palladium on charcoal, as for the synthesis of **31c**, to give **36** (57%) as a buff solid: mp >300°C; *m/z* 359.1196 (M + Na) ($C_{23}H_{16}NaN_2O$ requires 359.1160), 337.1331 (M + H) ($C_{23}H_{17}N_2O$ requires 337.1341.

5-Benzamido-4-phenylisoquinolin-1-one (37)

Compound **31a** (68 mg, 0.25 mmol) was stirred with benzoyl chloride (39 mg, 0.28 mmol) in pyridine (2.0 mL) at 90°C for 16 h. Evaporation and chromatography (ethyl acetate → ethyl acetate / methanol 4:1) gave **31** (31 mg, 36%) as a very pale pink solid: mp 230-232°C (decomp.); δ_H (CD_3OD) 7.10-7.16 (3 H, m, Ph 3,4,5-H_3), 7.24 (1 H, s, 3-H), 7.26-7.36 (4 H, m, 7-H and COPh 3,4,5-H_3), 7.62 (2 H, d, *J* 7.2, Ph 2,6-H_2), 7.71 (1 H, d, *J* 7.8, 6-H), 7.78 (1 H, d, *J* 7.4, COPh 2,6-H_2), 8.42 (1 H, d, *J* 7.8, 8-H); δ_C (CD_3OD) 119.5, 127.7, 127.6, 128.4, 128.6, 128.9, 129.2, 129.4, 129.9, 130.3, 130.9, 131.8, 133.0, 134.6, 140.2, 164.1, 168.3; *m/z* 363.1133 (M + Na) ($C_{22}H_{16}NaN_2O_2$ requires 363.1109), 341.1312 (M + H) ($C_{17}H_{15}N_2O_2$ requires 341.1290).

X-Ray crystallography

General: All data were collected at 150 K on a Nonius kappaCCD diffractometer. The structures were uniformly solved using SHELXS-97[23] and refined using full-matrix least squares in SHELXL-97.

Crystal data for **26a:** $C_{16}H_{12}N_2O_3$, *M* = 280.28, λ = 0.71073 Å, orthorhombic, space group = $P2_12_12_1$, *a* = 7.4710(1), *b* = 8.3050(1), *c* = 21.1940(4)Å, *U* = 1315.02(3) Å^3, *Z* = 4, D_c = 1.416 g cm^{-3}, μ = 0.100 mm^{-1}, *F*(000) = 584, crystal size = 0.50 × 0.40 × 0.30 mm. Reflections collected = 23576, unique reflections = 3016 [R_{int} = 0.0757], reflections observed (*I* > 2σ > (*I*)) = 2288, data / restraints / parameters = 3016/0/192. Final *R* indices [*I* > 2σ > (*I*)]; *R*1 = 0.0426, *wR*2 = 0.0893. *R* indices (all data); *R*1 = 0.0725, *wR*2 = 0.1022. Max / min peak and hole = 0.468, -0.407 eÅ^{-3}.

Crystal data for **33**: $C_{16}H_{11}BrN_2O_3$, *M* = 359.18, λ = 0.71073 Å, triclinic, space group = *P*-1 (No. 2), *a* = 8.6180(4), *b* = 9.9120(4), *c* = 10.2260(5)Å, α = 114.347(2), β = 108.553(2), γ = 100.738(2)°, *U* = 701.65(6) Å^3, *Z* = 2, D_c = 1.700 g cm^{-3}, μ = 2.944 mm^{-1}, *F*(000) = 360, crystal size = 0.40 × 0.30 × 0.30 mm. Reflections collected = 11401, unique reflections = 3168 [R_{int} = 0.0484], reflections observed (*I* > 2σ > (*I*)) = 2680, data / restraints / parameters = 3168/0/200. Final *R* indices [*I* > 2σ > (*I*)]; *R*1 = 0. 0303 , *wR*2 = 0. 0642. *R* indices (all data); *R*1 = 0. 0421 , *wR*2 = 0. 0680. Max / min peak and hole = 0.369, -0.388 eÅ^{-3}.

Crystal data for **35**: $C_{32}H_{24}N_2O_4$, *M* = 500.53, λ = 0.71073 Å, monoclinic, space group = *P*2/*c*, *a* = 13.9880(2), *b* = 10.9970(2), *c* = 16.1970(2)Å, *b* = 95.645(1)° *U* = 2479.44(7) Å^3, *Z* = 4, D_c = 1.341 g cm^{-3}, β = 0.089 mm^{-1}, *F*(000) = 1048, crystal size = 0.40 × 0.20 × 0.20 mm. Reflections collected = 44827, unique reflections = 5661 [R_{int} = 0.0507], reflections observed (*I* > 2σ > (*I*)) = 4556, data / restraints / parameters = 5661/0/350. Final *R* indices [*I* > 2σ > (*I*)]; *R*1 = 0. 0649 , *wR*2 = 0. 1729. *R* indices (all data); *R*1 = 0. 0836, *wR*2 = 0. 1860. Max / min peak and hole = 0.442, -1.102 eÅ^{-3}.

PARP-1 inhibition assay

Compounds were assayed for inhibition of the catalytic activity of PARP-1 using the FlashPlate scintillation proximity assay previously developed at KuDOS.[20] Compounds were evaluated at eight different concentrations in triplicate; data were fitted to the dose-response curve using a $\log_{10}$ concentration scale using SigmaPlot, IC_{50} values were measured in two or three independent experiments and the mean values are reported.

PARP-2 inhibition assay

Compounds were assayed for inhibition of the catalytic activity of PARP-2 using a method in which recombinant PARP-2 protein (recombinant) was bound down by a PARP-2-specific antibody in a 96-well white-walled plate. PARP-2 activity was measured following addition of ^{3}H-NAD$^+$ and DNA.[21] After washing, scintillant was added to measure the ^{3}H-incorporated. Compounds were evaluated at eight different concentrations in triplicate; data were fitted to the dose-response curve using a $\log_{10}$ concentration scale using SigmaPlot, IC_{50} values were measured in two or three independent experiments and the mean values are reported.

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Notes and references

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‡ Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre: CCDC 781105 (**26a**), CCDC 781106 (**33**) and CCDC 781107 (**35**). Requests for data should be addressed to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK.

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